IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants of: Voorberg, et al.

Group Art Unit: 1644

Serial No.: 09/674,752

Examiner: Maher M. Haddad

Filed: December 29, 2000

Docket: 294-86 PCT/US/RCE

For: METHOD FOR DIAGNOSIS AND TREATMENT OF HEMOPHILIA A PATIENTS WITH AN INHIBITOR

Dated: August 23, 2005

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	*I hereby certify this correspondence is being deposited with the United States Postal Service as first class mail, postpaid in an envelope, addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 20231 on August 23, 2005* *Dated: 8/23/05*

DECLARATION UNDER 37 C.F.R. 1.132 OF JOHANNES JACOBUS VOORBERG

Sir:

I, Johannes Jacobus Voorberg, Ph.D., declare as follows:

1. I am a co-inventor of the above-identified application.

2. I received a Ph.D. from the University of Amsterdam in 1991. I am an expert in the field of inhibitory antibodies directed to factor VIII Currently, I am a senior scientist at Sanquin Research. Attached as Exhibit 1 is my *curriculum vitae.*

JV 8/23/2005

3. I am a co-author of the Davies et al. reference (*Thromb. Haemostas.*, Supplement:2352, 1997) cited by the examiner in the Office Action of March 11, 2005. Davies et al. mentions that eight FVIII scFvs were selected by panning on immobilized rFVIII. In the Office Action, the examiner contends that the ability of the scFvs of Davies et al. to interfere with the activity of factor VIII inhibitors is an inherent property of the scFvs disclosed in Davies et al.

4. To demonstrate that the ability of a polypeptide to interfere with the activity of factor VIII inhibitors is **not** an inherent property of scFv polypeptides capable of specific binding to FVIII, the experiments described in exhibit 2 were performed by me or by persons directly under my supervision and control.

5. Briefly, a total of twenty different scFvs which bind to factor VIII were isolated. The twenty svFvs are listed in table 1 of exhibit 2. Twelve of the twenty scFvs were tested for their ability to neutralize (i.e., interfere with) factor VIII inhibitors. As shown in table 1, only five of the twelve tested scFvs were capable of neutralizing (i.e., interfere with) factor VIII inhibitors. See sample numbers 3, 16, 17, 18, and 19.

6. Therefore, not all svFv polypeptides that bind to factor VIII are capable of interference with factor VIII inhibitors. Accordingly, the ability of scFv to interfere with factor VIII inhibitors is not an inherent property of all scFvs which are capable of binding to factor VIII.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of

8/23/2005

the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Dated: 23/8/2005

Johannes Jacobus Voorberg, Ph.D.

Curriculum vitae Dr. J. Voorberg

Date of birth:	20-11-1961
University training:	University of Wageningen, School of Molecular Sciences
Ph.D. training:	Department of Molecular Biology under supervision of Prof. dr. H. Pannekoek en Dr. J.A. van Mourik (june 1987-june 1991)
Ph.D. thesis:	"Biosynthesis and assembly of human von Willebrand factor" (Nov 1991) University of Amsterdam.
Current position:	Senior Scientist at Department of Plasma Proteins, Sanquin Research, Amsterdam
Expertise:	Biology of haemostatic proteins with emphasis on factor VIII and von Willebrand factor. Cell biology of von Willebrand factor in endothelial cells constitutes an important line of investigation. During the last five years research on molecular biological aspects of factor VIII inhibitor formation has been studied using phage display and transgenic animal models. These lines of research have resulted in over 40 publications and the following 4 Ph D theses: "Consequences of haemophilia treatment" Karin Fijnvandraat (1998). "Small GTP binding proteins involved in regulated secretion of von Willebrand factor from endothelial cells" Hubert P.J.C. de Leeuw (2000). "Molecular analysis of factor VIII inhibitors using phage display" Edward N. van den Brink (2000). "Inhibitor formation in mild haemophilia A" Wendy S. Bril (2003).
Foreign training:	Massachusets Institute of Technology, Boston Department of Biochemistry (May-Dec, 1986) University of Minnesota, Minneapolis Department of Medicine, and Vascular Biology Center, University of Minnesota Medical School (Feb-July, 2004)
Research grants:	Netherlands Organization of Scientific Research (NWO), Netherlands Heart Foundation (NHS), Netherlands Thrombosis

Foundation (TSN), Landsteiner Foundation for Blood Transfusion Research (LSBR) and Amsterdam-Leiden-Institute-for-Immunology (ALIFI).

List of publications

1. Klugkist, J., **Voorberg, J.**, Haaker, H.and Veeger, C. (1987) Characterisation of three different flavodoxins from Azotobacter vinelandii. Eur. J. Biochem. 155, 33-40.

2. Pannekoek, H.and **Voorberg, J.** (1989) Molecular cloning, expression and assembly of multimeric von Willebrand factor. Baillieres Clin. Haematol. 2, 879-896.

3. **Voorberg, J.**, Fontijn, R., van Mourik, J.A. and Pannekoek, H (1990) Domains involved in multimer assembly of von Willebrand factor (vWF): multimerization is independent of dimerization. EMBO J. 9, 797-803.

4. Van de Ven, W.J., **Voorberg, J.**, Fontijn, R., Pannekoek, H., van den Ouweland, A.M., van Duijnhoven, H.L., Roebroek, A.J. and Siezen, R.J. (1990) Furin is a subtilisin-like proprotein processing enzyme in higher eukaryotes. Mol. Biol. Rep. 14, 265-270.

5. Leyte, A., **Voorberg, J.**, van Schijndel, H.B., Duim, B., Pannekoek, H. and van Mourik, J.A. (1991) The pro-polypeptide of von Willebrand factor is required for the formation of a functional factor VIII-binding site on mature von Willebrand factor. Biochem. J. 274, 257-261.

6. **Voorberg, J.**, Fontijn, R., Calafat, J., Janssen, H., van Mourik, J.A. and Pannekoek, H. (1991) Assembly and routing of von Willebrand Factor variants: the requirements for disulfide-linked dimerization reside within the carboxy-terminal 151 amino acids. J. Cell Biol. 113, 195-205.

7. Ribba, A.S., **Voorberg, J.**, Meyer, D., Pannekoek, H. and Pietu, G. (1992) Characterization of recombinant von Willebrand factor corresponding to mutations in type IIA and type IIB von Willebrand disease. J. Biol Chem. 267, 23209-23215.

8. **Voorberg, J.**, Fontijn, R., Calafat, J., Janssen, H., van Mourik, J.A. and Pannekoek, H. (1993) Biogenesis of von Willebrand factor-containing organelles in heterologous transfected CV-1 cells. EMBO J. 12, 749-758.

9. **Voorberg, J.**, Roelse, J., Koopman, R., Büller, H., Berends, F., ten Cate, J.W., Mertens, K. and van Mourik, J.A. (1994) Association of idiopathic venous thromboembolism with single point-mutation at Arg^{506} of factor V. The Lancet 343, 1535-1536.

10. Spaargaren, J., Giesen, P.L.A., Janssen, M.P., **Voorberg, J.**, Willems, G.M. and van Mourik, J.A. (1995) Binding of blood coagulation Factor VIII and its light chain to phosphatidylserine/phosphatidylcholine bilayers as measured by ellipsometry. Biochem. J. 310, 539-545.

11.Donath, M-J.S.H., de Laaf, R.T.M., Biessels, P.T.M., Lenting, P.J., van de Loo, J-W., van Mourik, J.A., **Voorberg, J.** and Mertens, K. (1995) Characterization of des-(741-1668)-factor VIII, a single-chain factor VIII variant with a fusion site susceptible to proteolysis by thrombin and factor Xa. Biochem. J. 312, 49-55.

12.Roelse, J.C., Koopman, M.M.W., Büller, H.R., ten Cate, J.W., Montaruli, B., van Mourik, J.A. and **Voorberg, J.** (1996) Absence of mutations at the activated protein C cleavage sites of factor VIII in 125 patients with venous thrombosis. Brit. J. Haematol. 92, 740-743.

13.**Voorberg, J.**, van Stempvoort, G., Klaasse Bos, J.M., Mertens, K., van Mourik, J.A. and Donath, M.J.S.H. (1996) Enhanced thrombin sensitivity of a factor VIII-heparin cofactor II hybrid. J. Biol. Chem. 271, 20985-20988.

14.Montaruli, B, **Voorberg, J.** Tamponi, G. Borchiellini, A. Muleo, G. Pannocchia, A. van Mourik, J.A. Schinco, P. (1996) Arterial and venous thrombosis in two Italian families with the factor V $Arg^{506} \rightarrow Gln$ mutation. Eur. J. Haematol, 57, 96-100.

15.Montaruli, B., Borchiellini, A., Giorda, L., Bessone, P., van Mourik, J.A., **Voorberg, J.**, Schinco, P (1996) Factor V $Arg^{506} \rightarrow Gln$ mutation in patients with antiphospholipid antibodies. Lupus 5, 303-306.

16.**Voorberg, J.**, de Laaf, R.T.M., Koster, P.M. and van Mourik, J.A. (1996) Intracellular retention of a factor VIII protein with an $Arg^{2307} \rightarrow Gln$ mutation as a cause of haemophilia A. Biochem. J. 318, 931-937.

17.Borchiellini, A., Fijnvandraat, K., ten Cate, J.W., Pajkrt, D., van Deventer, S.J.H., Pasterkamp, G., Meijer-Huizinga F., Zwart-Huinink, L., **Voorberg, J.**, and van Mourik, J.A. (1997) Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D arginine vasopressin in humans. Blood 88, 2951-2958.

18.Fijnvandraat, K., Turenhout, E.A.M., Van den Brink, E., ten Cate, J.W., van Mourik, J.A., Peters, M. and **Voorberg, J.** (1997) The missense mutation $Arg^{593} \rightarrow Cys$ is related to antibody formation in a patient with mild hemophilia A. Blood 89, 4371-4377.

19.Fijnvandraat, K., Celie, P.H.N., Turenhout, E.A.M., Cate ten, J.W., van Mourik, J.A. Mertens, K, Peters, M. and **Voorberg, J.** (1998) A human alloantibody interferes with binding of factor IXa to the factor VIII light chain. Blood 91, 2347-2353.

20.de Leeuw, H.PJ.C., Koster, P.M., Calafat, J., Janssen, H., van Zonneveld A-J., van Mourik, J.A. and **Voorberg, J.** (1998) Small GTP-binding proteins in human endothelial cells. Brit. J. of Haematol. 103, 15-19.

21. Van den Brink, E.N., Timmermans, S.M.H., Turenhout, E.A.M., Bank, C.M.C., Fijnvandraat, K., **Voorberg, J.** and Peters, M. (1999) Longitudinal analysis of factor VIII inhibitors in a previously untreated mild haemophilia A patient with an $Arg^{593} \rightarrow$ Cys substitution. Thromb. Haemost. 81, 723-726.

22. De Leeuw, H.P.J.C., Wijers-Koster, P.M., van Mourik J.A. and **Voorberg, J.** (1999) Small GTP-binding protein RalA associates with Weibel-Palade bodies in endothelial cells. Thromb. Haemost. 82, 1177-1181.

23. Van den Berg, H.M., Roosendaal, G., **Voorberg, J.** and Mauser-Bunschoten, E.P. (1999) Inhibitor development in a multitransfused patient with severe haemophilia A. Thromb. Haemost. 82, 151-152 (letter to the editor).

24. Van den Brink, E.N., Turenhout, E.A.M., Davies, J., Bovenschen, N., Fijnvandraat, K., Ouwehand, W.H., Peters, M. and **Voorberg, J.** (2000) Human antibodies with specificity for the C2 domain of factor VIII are derived from VH1 germline genes. Blood 95, 558-563.

25. Roelse J.C., de Laaf R.T.M., Timmermans, S.M.H., Peters, M., van Mourik, J.A. and **Voorberg, J.** (2000) Intracellular accumulation of factor VIII induced by missense mutations $Arg^{593} \rightarrow$Cys and $Asn^{618} \rightarrow$Ser explains cross-reacting material-reduced haemophilia A. Brit. J. Haematol. 108, 241-246.

26. Van den Brink, E.N., Turenhout, E.A.M., Bank, C.M.C., Fijnvandraat, K., Peters, M. and **Voorberg J.** (2000) Molecular analysis of human anti-factor VIII antibodies by V gene phage display identifies a new epitope in the acidic region following the A2 domain. Blood 96, 540-545.

27. **Voorberg, J.** and Van den Brink, E.N. (2000) Phage display technology; a tool to explore the diversity of inhibitors to blood coagulation factor VIII. Seminars in Thrombosis and Haemostasis, 26, 143-150.

28. Van den Brink, E.N., Turenhout, E.A.M., Wijn-Maas, E.C.M., van der Meer, F.J.M., **Voorberg, J.** and Bosch L.J. (2000) Disappearance of factor VIII autoantibodies preceding autoimmune haemolytic anemia. Haemophilia, 6, 698-701.

29. Echard, A., Obdam, F.J., De Leeuw, H.J.P.C., Jollivet, F., Savelkoul, P., Hendriks, W., **Voorberg, J.**, Goud, B. and Franssen, J.A.M. (2000) Alternative splicing of the human Rab6A gene generates two close but functionally different isoforms. Mol.Biol.Cell, 11, 3819-3833.

30. Van den Brink, E.N., Turenhout, E.A.M., Bovenschen, N., Heijnen, B.G.A.D.H., Mertens, K., Peters, M. and **Voorberg, J.** (2001) Multiple VH genes are used to

assemble human antibodies directed towards the A3-C1 domains of factor VIII. Blood 97, 966-972.

31.De Leeuw, H.J.P.C., Fernandez-Borja, M., Reits, E.A.J., Wijers-Koster, P.M., Neefjes, J., van Mourik, J.A. and **Voorberg, J.** (2001) Small GTP binding protein Ral mediates regulated exocytosis of von Willebrand factor by endothelial cells. Arterioscler Thromb Vasc Biol. 21, 899-904.

32.Vlot, A.J., Wittebol, S., Strengers, P.F.W., Turenhout, E.A.M., **Voorberg, J.**, van den Berg, M.H. and Mauser-Bunschoten, E.P. (2002) Factor VIII inhibitor in a mild haemophilia A patient with an Asn^{618}→Ser mutation responsive to a combination of immune tolerance induction and cyclophosphamide. Brit. J. Haematol. 117, 136-140.

33.Van Mourik, J.A., Romani de Wit, T., and **Voorberg, J.** (2002) Biogenesis and exocytosis of Weibel Palade bodies. Histochemistry and Cell Biology, 117, 113-122.

34.Bril, W.S., Turenhout, E.A.M., van den Brink, E.N., Kaijen, P.H.P., Peters, M. and **Voorberg, J.** (2002) Analysis of factor VIII inhibitors in a haemophilia A patient with an Arg^{593}→Cys mutation using phage display. Brit. J. of Haemat., 119, 393-396.

35.Van den Brink, E.N., Bril, W.S., Turenhout, E.A.M., Zuurveld, M., Bovenschen, N., Peters, M., Yee, T.T., Mertens, K., Lewis, D.A., Ortel, T.L., Lollar, P., Scandella, D., and **Voorberg, J.** (2002) Two classes of germline genes both derived from the VH1 family direct the formation of human antibodies that recognize distinct antigenic sites in the C2 domain of factor VIII. Blood 99, 2828-2834.

36.Romani de Wit, T., de Leeuw, H.P.J.C., Rondaij M.G., de Laaf R.T.M., Sellink E, Brinkman H-J, **Voorberg, J.** and van Mourik, J.A. (2003) Von Willebrand factor targets IL-8 to Weibel-Palade bodies. Exp Cell Research 268, 67-74.

37.Fijnvandraat K, Bril W.S., and **Voorberg, J.** (2003) Immunobiology of inhibitor development in haemophilia A. Seminars in Thrombosis and Haemostasis, 29, 61-68.

38.Bovenschen N, Boertjes RC, van Stempvoort G, **Voorberg J**, Lenting PJ, Meijer AB and Mertens K. (2003) Low-density lipoprotein receptor-related protein and factor IXa share structural requirements for binding to the A3 domain of coagulation factor VIII. J. Biol. Chem. 278, 9370-9377.

39.Romani de Wit, Rondaij MG, Hordijk PL, **Voorberg J** and van Mourik JA. (2003) Real-time imaging of the dynamics and secretory behaviour of Weibel-Palade bodies. Arterioscler Thromb Vasc Biol, 23, 755-761.

40.Astermark J, **Voorberg J**, Lenk H, DiMichele D, Shapiro A, Tjonnford G and Berntorp E. (2003) Impact of inhibitor epitope profile on the neutralizing effect agianst

plasma-derived and recombinant factor VIII concentrates in vitro. Haemophilia, 9, 567-572.

41.Schatz S, Turecek PL, Fiedler C, Zimmermann K, Gritsch H, **Voorberg J**, Schwarz HP, Dorner F and Scheiflinger F (2003) Evaluation of the haemostatic potential of factor VIII-heparin cofactor II hybrid proteins in a mouse model. Br. J. Haematol. 123, 692-695.

42.Rondaij MG, Sellink E, Gijzen KA, ten Klooster JP, Hordijk PL, van Mourik JA and **Voorberg J**. (2004) Small GTP binding protein Ral is invovled in cAMP-mediated release of von Willebrand factor from endothelial cells. Arterioscler Thromb Vasc Biol. 24, 1315-1320.

43.Bril WS. MacLean PE, Kaijen PH, van den Brink EN, Lardy NM, Fijnvandraat K, Peters M and **Voorberg J.** (2004) HLA class II genotype and factor VIII inhibitors in mild haemophilia A patients with an Arg593 to Cys mutation. Haemophilia 10, 509-514.

44.Luken BM, Turenhout EAM, Hulstein JJ, van Mourik JA, Fijnheer R and **Voorberg J.** (2005) The spacer domain of ADAMTS13 contains amajor bindig site for antiboides in patients with thrombotic thrombocytopenic purpura. Thromb Haemost 93, 267-274.

45.Lewis DA, Bovenschen N, Mertens K, **Voorberg J** and Ortel TL. (2005) Phospholipid vesicles interfere with the binding of antibody fragments to the light chain of factor VIII. Thromb Haemost 93, 833-841.

Twenty scFvs that bind to factor VIII were isolated. The isolated scFvs' ability to inhibit factor VIII was determined as follows:

100 µl of NP is diluted in 100 µl 100 mM Tris-HCl pH=7.4; 0.4% HSA. Next, 200 µl of different dilutions of scFv in 50 mM Tris-HCl pH=7.4; 0.2% HSA is added and incubated for 2 hour at 37°C. After incubation, residual factor VIII activity is determined is measured using a Sysmec C7000 (source of hemophilia A plasma: Dade Behring, OTXW17; Actin®FSL Activated PTT reagens; Dade Behring 52731334). Three different dilutions are measured per concentration of scFv. The ability of the scFvs to inhibit factor VIII is listed in table 1 below.

As shown in table 1, scFvs sample numbers 1, 5 and 7 inhibited factor VIII activity. The remaining scFvs samples (i.e., sample numbers 2-4, 6, 8-20) did not inhibit factor VIII activity.

The ability of scFv to interfere with the activity of factor VIII inhibitors was assayed as follows:

100 µl of NP is diluted in 100 µl 100 mM Tris-HCl pH=7.4; 0.4% HSA. Next, 150 µl of different dilutions of scFv in 50 mM Tris-HCl pH=7.4; 0.2% HSA are added and for incubated for 1 hour at 37°C. After incubation, 50 µl of diluted monoclonal antibody or diluted patient plasma is added till a final concentration of 2 BU/ml. The mixture is incubated for 1 hour at 37°C. Total volume is now 400 µl. Sample is incubated for 1 hour and then residual factor VIII activity in the incubation mixture is measured using a Sysmec C7000 (source of hemophilia A plasma: Dade Behring, OTXW17; Actin®FSL Activated PTT reagens; Dade Behring 52731334). Three different dilutions are measured per concentration of scFv.

As shown in table 1, scFvs sample numbers 3, 16, 17, 18, and 19 are capable of interfering with factor VIII inhibitors, but do not inhibit factor VIII activity.

JV
23/08/2005

Therefore, the ability of scFvs to interfere with factor VIII inhibitors is not an inherent property of scFvs to bind to factor VIII.

Table 1: Properties of factor VIII scFvs.

Sample No.	scFv Name	epitope	VH segment	Inhibition of Factor VIII	Interference with Factor VIII Inhibitors
1	VK34	A2	DP10	positive	negative
2	VK41	A2	DP47	negative	not tested
3	WP102	A2	DP47	negative	positive
4	WP137	A2	DP58	negative	not tested
5	B35	A3-C1	DP49	positive	negative
6	B04	A3-C1	DP77	negative	not tested
7	B38	A3-C1	DP15	positive	negative
8	KM37	A3-C1	DP14	negative	negative
9	KM38	A3-C1	DP49	negative	not tested
10	KM36	A3-C1	DP77	negative	not tested
11	WR1	C2	DP5	negative	negative
12	WR16	C2	DP5	negative	negative
13	WR17	C2	DP5	negative	negative
14	VK29	C2	DP25	negative	not tested
15	VK33	C2	DP79	negative	not tested
16	EL5	C2	DP14	negative	positive
17	EL14	C2	DP10	negative	positive
18	EL9	C2	DP88	negative	positive
19	IT2	C2	DP14	negative	positive
20	WR2	C2	DP88	negative	not tested

JV

23/08/2005